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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Dales
Serial No:	09/265,926
For:	Preparation of Purines
Art Unit No.:	1611
Examiner:	Mark L Berch

DECLARATION OF ALAN JONES

I, Alan Jones, hereby declare:

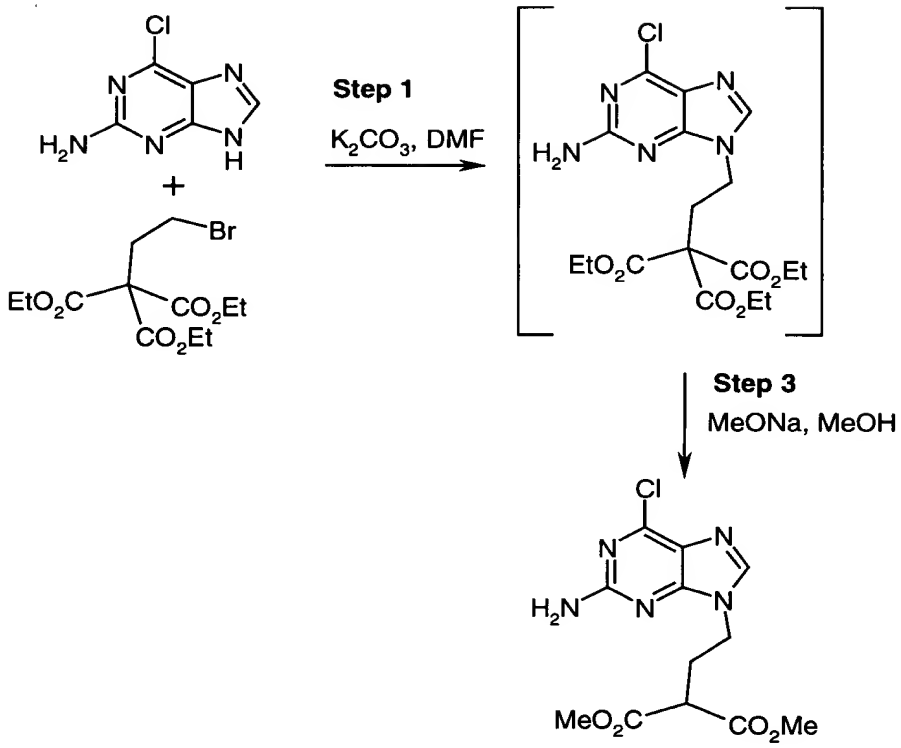
1. That I am Alan Jones of SmithKline Beecham p.l.c., Tonbridge, United Kingdom. I studied for a Bachelor of Science in Chemistry (1989) and a PhD in Organic Chemistry (1993) at the University of Manchester. Since 1993 I have been working for the pharmaceuticals business of SmithKline Beecham p.l.c. as an organic chemist within chemical development. At the current time I am an Investigator in the Synthetic Chemistry department. I am author or co-author of over 10 publications and presentations relating to organic chemistry.

2. I have read and understood the present application US Serial No. 09/265,926 ('926), which relates to a process for the production of purines, e.g. famciclovir and penciclovir. I have also read and understood European Patent Application No. 302644 ('644) filed 25 July 1988.

3. I understand that the process claimed in '926 differs from that exemplified in '644 in that the 6-chloro substituent is removed after the esterification of the 4-hydroxy-3-hydroxymethylbut-1-yl groups rather than after the coupling of triethyl 3-bromopropane-1,1,1-tricarboxylate to 2-amino-6-chloropurine.

4. The process described in '926 Examples 1-3(a) is shown below as Stage 1, steps 1 and 3 and Stage 2, steps 1 to 3 verbatim:

Stage 1



Step 1

Reaction of 2-amino-6-chloro purine with 4-bromo-2,2-bisethoxycarbonyl butyric acid ethyl ester

i) Mixture of 2-amino-6-chloro purine (9.18g, 53.1 mmol), triethyl 3-bromopropane-1,1,1-tricarboxylate (20.33g, 57.3 mmol), potassium carbonate (11.1 g, mmol) and N,N-dimethyl formamide (190 ml) were stirred together at 60-63°C for 22 hours.

ii) The hot reaction mixture was filtered through a bed of celite and the cake washed with DMF (30 ml).

iii) The combined filtrate and washings was evaporated by high vacuum distillation to give a red brown coloured oil.

Step 3

Decarboxylation and transesterification of triester

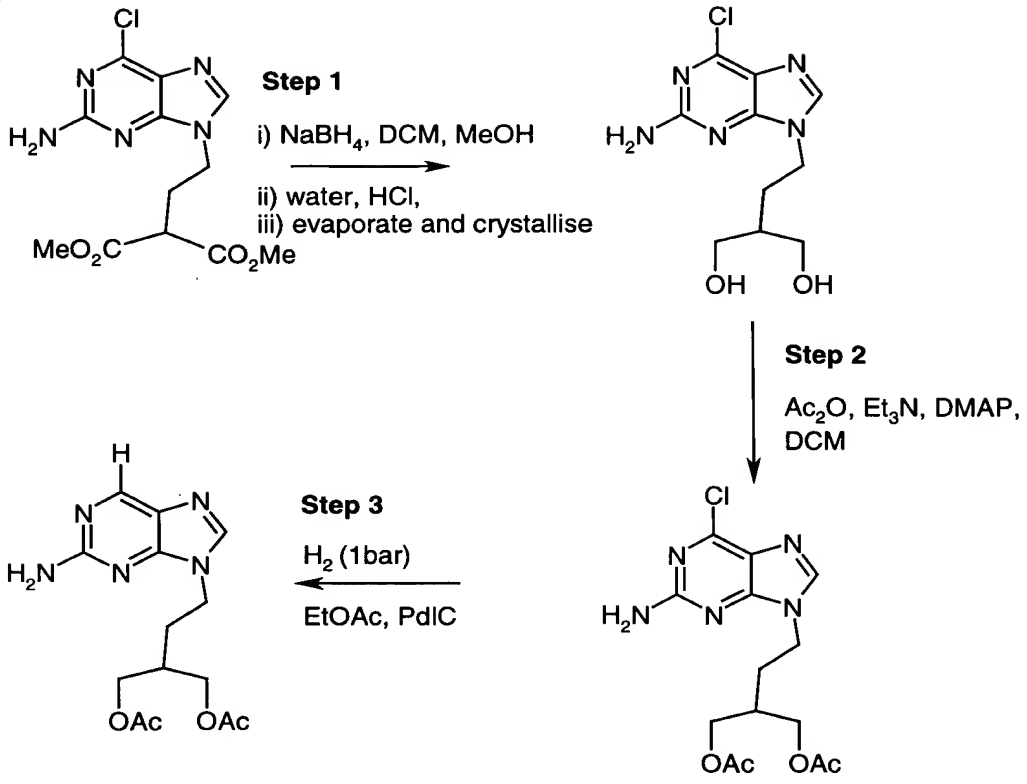
i) The product from **Step 1** was dissolved in methanol (140 ml) at 20°C and then a solution of sodium methoxide (1.20 g) in methanol (40 ml) was added with stirring.

ii) After 20 minutes a precipitate formed.

iii) The reaction mixture was cooled to 15°C and held at this temperature for 30 minutes

iv) The product was isolated by filtration and washed with methanol (10 ml) and dried at 40°C under vacuum. Weight yield 12.0 g of 95% purity.

Stage 2



Step 1

Reduction to diol

i) A mixture of 2-amino-9-(methyl 2-carbomethoxybutanoate-4-yl) purine (32.7 g), sodium borohydride (11.5 g) and dichloromethane (125 ml) was stirred at 20°C.

ii) Methanol (75 ml) was added over 2 hours maintaining a reaction temperature of 20-22°C

iii) The reaction mixture was left to stir for a further 1.5 hours.

iv) Water (100 ml) was added.

v) Concentrated hydrochloric acid (20-22 ml) was added dropwise to pH=6.7-7.0.

vi) Dichloromethane and methanol were removed by evaporation under vacuum until a volume of 150 ml remained.

vii) The precipitate was filtered and the cake washed with cold water (20 ml). Weight yield 30-40g.

Step 2

Acetylation of diol

i) The wet cake from **Step 1** (30-40g) was stirred with triethylamine (15 ml) and 4-N,N-dimethylamino pyridine (1 g) in dichloromethane (250 ml).

ii) Acetic anhydride (75 ml) was added dropwise over 20 to 30 minutes at such a rate to control the reflux.

iii) The reaction mixture was heated at reflux temperature for a further 1.5 hours.

iv) The reaction mixture was cooled to 20 °C and neutralised to pH=6.4-6.5 with 20% w/w sodium hydroxide solution.

v) The dichloromethane layer was separated and the aqueous phase extracted with dichloromethane (100 ml).
vi) The combined dichloromethane phases were evaporated to dryness.
vii) The crude damp solid was recrystallised from 3:1 methanol:water (75 ml), cooling the precipitate to 5°C for 1 hour before filtration.
viii) The product was washed with cold (0°C) 3:1 methanol:water (5 ml) and dried at 40°C in a vacuum oven. Weight yield 23g of 97-98% purity.

Step 3

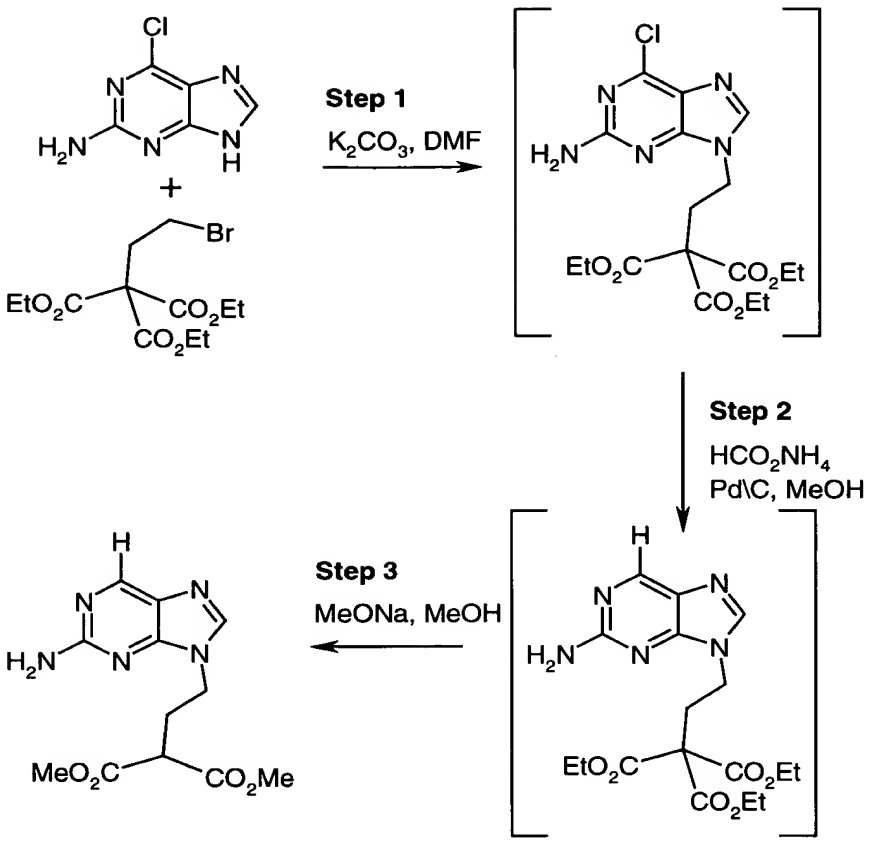
Hydrogenation of chloro purine

i) A mixture of 9-(4-acetoxy-3-acetoxymethylbut-yl)-2-aminopurine (15.4g), 5% palladium on carbon (6.16 g), triethylamine (6.6 ml) and ethyl acetate (77 ml) was stirred at 50°C under a hydrogen atmosphere at 1 bar pressure in an autoclave for 3-5 hours.
ii) The reaction mixture was washed out of the autoclave with ethyl acetate (30 ml) keeping the washings at 50°C.
iii) The reaction mixture and washings were filtered through a celite bed and the filter bed washed with ethyl acetate (30 ml).
iv) The combined filtrate and washings were evaporated to dryness to give a crude white solid.
v) The solid was recrystallised form n-butanol (62 ml), stirring the cooled solution at 0-5°C for 3 hours before filtration.
vi) The product was filtered off and washed with the mother liquors.
vii) The solid was re-slurried in n-heptane (50 ml), stirred for 30 minutes and filtered.
viii) The product was dried at 40°C for 16 hours under vacuum. Weight yield 11-11.3g.

5. I have now repeated the process described in '926 Examples 1-3(a) this time removing the 6-chloro as described in '644 after the coupling of triethyl 3-bromopropane-1,1,1-tricarboxylate to 2-amino-6-chloropurine (Stage 1, step 2), rather than after the esterification of the 4-hydroxy-3-hydroxymethylbut-1-yl groups (Stage 2, step 3). This process, which is referred to as the "EP302644 Type Process" differs from that described in '926 only at the dechlorination step, the remaining steps in the process use exactly the same reaction conditions as '926 thus allowing a direct comparison of the '926 and EP302644 Type Process.

6. The experimental details used for the EP302644 Type Process described in paragraph 5 is shown below as Stage 1, steps 1 to 3 and Stage 2, steps 1 and 2:

Stage 1



Step 1

Reaction of 2-amino-6-chloro purine with 4-bromo-2,2-bisethoxycarbonyl butyric acid ethyl ester

i) A mixture of 2-amino-6-chloro purine (9.18g, 53.1 mmol), triethyl 3-bromopropane-1,1,1-tricarboxylate (20.33g, 57.3 mmol), potassium carbonate (11.1 g, 80.3 mmol) and N,N-dimethyl formamide (190 ml) were stirred together at 60 to 63°C for 22 hours.

ii) The hot reaction mixture was filtered through a bed of celite and the cake washed with N,N-dimethyl formamide (30 ml).

iii) The combined filtrate and washings was evaporated by high vacuum distillation to give a red brown coloured oil. Weight yield 32.23 g.

Step 2

Hydrogenation of chloro purine

i) A mixture of crude 2-amino-6-chloro-9-(ethyl 2,2-dicarboethoxybutanoate-4-yl) purine (32 g) prepared in **Step 1**, ammonium formate (30 g) and 5% palladium on carbon (6g) in methanol was heated at reflux under nitrogen for 2 hours.

ii) The reaction mixture was cooled.

iii) The mixture was filtered and the filtrate evaporated.

iv) The residues were dissolved in water (600 ml) and extracted with chloroform (3 x 300 ml) and the combined extracts dried over magnesium sulfate.

v) The drying agent was removed by filtration and the filtrate evaporated to give the product as a viscous yellow oil. Weight yield 17.87 g.

Step 3

Decarboxylation of triester

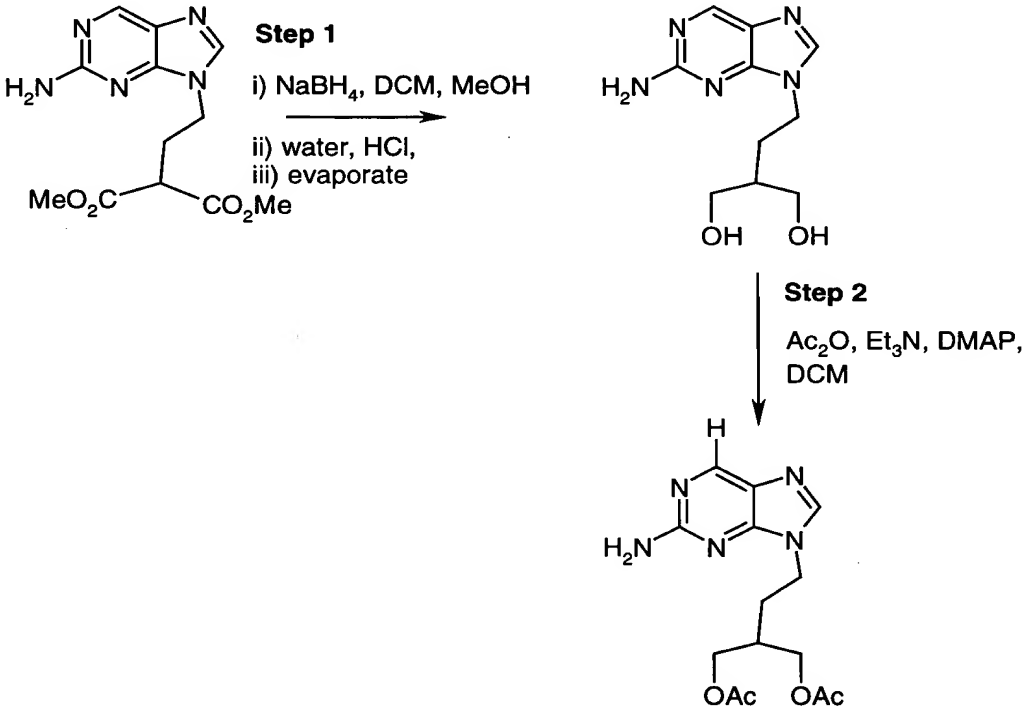
i) The product from **Step 2** (17.50 g) was dissolved in methanol (140 ml) at 20°C and then a solution of sodium methoxide (1.20 g) in methanol (40 ml) was added with stirring.

ii) The stirring was continued for 1 hour.

iii) No product crystallised from the reaction mixture. The reaction was a homogeneous yellow solution.

iv) The reaction mixture was evaporated to give a crude yellow coloured oily solid. Weight yield 14.79 g.

Stage 2



Step 1

Reduction of dimethyl ester.

i) A mixture of 2-amino-6-chloro-9-(methyl 2-carbomethoxybutanoate-4-yl) purine (14 g), sodium borohydride (4.90 g) and dichloromethane (54 ml) was stirred at 20°C.

ii) Methanol (32 ml) was added slowly over 2 hours maintaining a reaction temperature of 20-22°C with intermittent cooling with an ice/water bath.

iii) The reaction mixture was left to stir at ambient temperature for a further 1.5 hours.

iv) Water (43 ml) was added.

v) The reaction mixture was neutralised by dropwise addition of concentrated hydrochloric acid (approx. 9 ml) to pH=6.9 maintaining an internal temperature of 20-22°C.
vi) Dichloromethane and methanol were removed by evaporation under vacuum until a volume of 65 ml was obtained.
vii) The mixture was cooled to 5°C and stirred at this temperature for 30 minutes.
viii) The reaction mixture was stirred at 0-5°C for a further 1 hour.
ix) No precipitate formed and the reaction mixture was evaporated to dryness to give a crude yellow coloured solid. Weight yield 21.06 g.

Step 2
Acetylation of diol.
i) The crude solid from **Step 1** (20.5 g) was stirred with triethylamine (6.5 ml) and 4-N,N-dimethylamino pyridine (0.43 g) in dichloromethane (110 ml).
ii) Acetic anhydride (32 ml) was added dropwise over 20 minutes.
iii) The reaction mixture was heated at reflux temperature for a further 1.5 hours.
iv) The reaction mixture was cooled to 20 °C and neutralised to pH=6.4 with 20% w/w sodium hydroxide solution.
v) The dichloromethane layer was separated and the aqueous phase extracted with dichloromethane (45 ml).
vi) The combined dichloromethane phases were evaporated to dryness.
vii) The resulting crude oil did not crystallise from n-butanol or from methanol/water mixtures.

7. The processes of paragraphs 4 and 6 are summarised for comparative purposes in the Annex.

8. The overall yield of the process of '926 is 41% of a crystalline solid representing a 41% yield of usable famciclovir, i.e. famciclovir of a pharmaceutically acceptable quality. In comparison the overall yield of the corresponding process wherein the 6-chloro substituent is removed after the coupling of triethyl 3-bromopropane-1,1,1-tricarboxylate to 2-amino-6-chloropurine is 14% of a crude brown oil representing a 0% yield of usable famciclovir.

9. These experimental data confirm that the continued presence of the 6-chloro substituent during decarboxylation and through to the final step of the process is responsible for the advantages of the process of '926 over that described in '644 rather then the particular reaction conditions employed such as removal of the column chromatography steps or the nature of the ester obtained following decarboxylation of the compound of formula (VI).

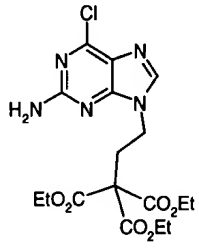
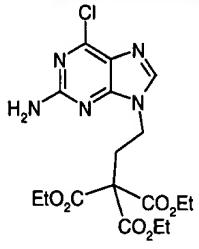
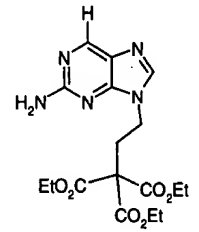
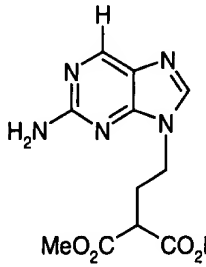
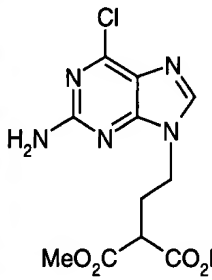
10. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the

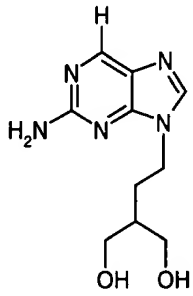
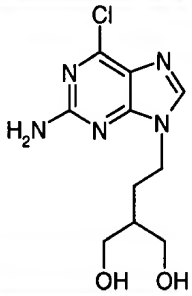
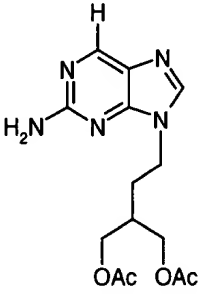
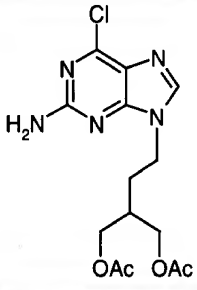
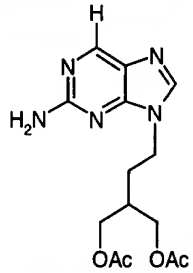
United States Code and that such wilful false statements may jeopardise the validity of this application or any patent issuing thereon.

Date: 20th December 1999

ALAN JONES

ANNEX

	"EP 302644 Type Process"			US 09/265,926		
Stage Number	Product	Comments		Product	Comments	
Stage 1 Step 1		Yield	32.23 g		Yield	Not given
		Form	Red/brown oil		Form	Red oil
Step 2		Yield	17.87 g	Not Applicable		
		Form	Viscous oil			
Step 3		Yield	14.79 g		Yield	12.0 g (65%)
		Form	Yellow oily solid		Form	Crystalline solid
	N9 and N7 were not separated. Purity Approx 45% by NMR				Assay 95%, selective crystallisation of N9 isomer	

Stage 2 Step 1		Yield	21.06 g		Yield	Quantitative wet cake
		Form	Yellow oily solid		Form	Wet cake
		Evaporated to dryness. No purification by precipitation/washing possible			Cake washed with water to remove impurities	
Step 2		Yield	8.31 g		Yield	70%
		Form	Brown oil		Form	Crystalline solid
		Famciclovir as a crude brown oil. Purity Approx. 30% by NMR.			Product of high purity (98%) and utilisable form for a pharmaceutical intermediate	
Step 3	Not Applicable				Yield	90%
					Form	Crystalline solid
					Famciclovir of pharmaceutical acceptable quality	
Overall Yield	0% (usable famciclovir) (weight yield 14%)			41% (usable famciclovir)		
Form	Crude Oil			Crystalline solid		